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Search Results - Record(s) 1 through 2 of 2 returned.

L8: Entry 1 of 2

File: USPT

Oct 9, 2001

US-PAT-NO: 6299881

DOCUMENT-IDENTIFIER: US 6299881 B1

TITLE: Uronium salts for activating hydroxyls, carboxyls, and polysaccharides, and conjugate vaccines, immunogens, and other useful immunological reagents produced using uronium salts

DATE-ISSUED: October 9, 2001

US-CL-CURRENT: 424/194.1, 424/146.1, 424/178.1, 424/193.1, 424/196.11, 424/197.11, 424/201.1,
424/202.1, 424/203.1, 424/236.1, 424/239.1, 424/240.1, 424/244.1, 424/256.1, 424/280.1, 435/188,
435/961, 435/964, 436/543, 514/54, 530/391.1, 530/403, 530/404, 530/405, 530/406, 530/409,
530/411, 530/806

INT-CL: [7] A61 K 39/385, A61 K 39/02, C07 K 17/10, C07 D 487/00

L8: Entry 2 of 2

File: USPT

Sep 14, 1999

US-PAT-NO: 5952454

DOCUMENT-IDENTIFIER: US 5952454 A

TITLE: Linking compounds useful for coupling carbohydrates to amine-containing carriers

DATE-ISSUED: September 14, 1999

US-CL-CURRENT: 528/332, 525/54.1, 528/339.3

INT-CL: [6] C08 G 69/26

WEST Search History

DATE: Thursday, April 18, 2002

Set Name Query

side by side

DB=USPT; PLUR=YES; OP=AND

		<u>Hit Count</u>	<u>Set Name</u>
		result set	
L1	vi and adh	597	L1
L2	L1 and (linker or linked or link or conjugate or conjugated)	408	L2
L3	L1 and salmonell\$	38	L3
L4	adipic or hyrazide or dihydrazide	35123	L4
L5	L4 and vi	7751	L5
L6	L5 and (reta or eta or exotoxin or exo-toxin or pseudomonas)	417	L6
L7	L6 and salmonell\$	36	L7
L8	('6299881' '5952454')[PN]	2	L8

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L7: Entry 6 of 36

File: USPT

Sep 14, 1999

DOCUMENT-IDENTIFIER: US 5952454 A

TITLE: Linking compounds useful for coupling carbohydrates to amine-containing carriers

Brief Summary Paragraph Right (5):

A further known procedure employs adipic acid dihydrazide as a homobifunctional spacer. In the adipic acid dihydrazide method, the carboxylic acid group of a carbohydrate or polysaccharide is coupled to adipic acid dihydrazide in the presence of a water-soluble carbodiimide by way of the hydrazide linkage. The spacer is terminated by a strongly nucleophilic hydrazino group which can be coupled to the carboxylic acid group of a protein.

Brief Summary Paragraph Right (8):

Linkers or spacer compounds are also described by Fattom et al. *Immunity*, February 1992, pp. 584-589. The specific linkers disclosed by Fattom et al. are adipic acid dihydrazide and N-succinimidyl-3-(2-pyridylidithio) propionate. This article relates to the formation of conjugates of *Staphylococcus aureus* Type 8 capsular polysaccharide. The bond formed between these linkers and the capsular polysaccharide are identical. However, the N-hydroxysuccinimyl moiety reacts mostly with lysine amine groups of the protein while the hydrazide binds to the carboxyl in the protein.

Brief Summary Paragraph Right (11):

The most successful of all carbohydrate pharmaceuticals so far have been the carbohydrate based, antibacterial vaccines [1]. The basis of using carbohydrates as vaccine components is that the capsular polysaccharides and the O-specific polysaccharides on the surface of pathogenic bacteria are both protective antigens and essential virulence factors. The first saccharide-based vaccines contained capsular polysaccharides of *Pneumococci*: in the United States a 14-valent vaccine was licensed in 1978 followed by a 23-valent vaccine in 1983. Other capsular polysaccharides licensed for human use include a tetravalent meningococcal vaccine and the Vi polysaccharide of *Salmonella typhi* for typhoid fever. The inability of most polysaccharides to elicit protective levels of anti-carbohydrate antibodies in infants and adults with weakened immune systems could be overcome by their covalent attachment to proteins that conferred T-cell dependent properties [2]. This principle led to the construction of vaccines against *Haemophilus influenzae* b (Hib) [3] and in countries where these vaccines are routinely used, meningitis and other diseases caused by Hib have been virtually eliminated. [4] Extension of the conjugate technology to the O-specific polysaccharides of Gram-negative bacteria provided a new generation of glycoconjugate vaccines that are undergoing various phases of clinical trials [5].

Brief Summary Paragraph Right (40):

The compound of formula (II) is deprotected sequentially first in the carbohydrate moiety and then in the aldehyde function to create an aldehyde functionality at the end of the aglycon moiety by known means to give

an aldehyde of the general formula IV: ##STR6## in which R.sub.1, R.sub.2, R.sub.3, R'.sub.3, R.sub.4, R.sub.5, m and n have the meanings given above. Aldehydes of formula (IV) are then reacted with a carrier having at least one primary amino group of the general formula (III) to give a material of the general formula (V): ##STR7## in which G, R.sub.1, R.sub.2, R.sub.4, R.sub.5, R.sub.6, n and m have the meanings given above. Reduction of the material of formula (V) provides a product of the general formula (VI): ##STR8## in which G, R.sub.1, R.sub.2, R.sub.4, R.sub.5, R.sub.6, n and m have the meanings given above.

Brief Summary Paragraph Right (52):

Trial carriers include bovine serum albumin, and chicken serum albumin. Examples of carriers for vaccine are natural peptides and proteins such as diphtheria toxoid, tetanus toxoid, Pseudomonas aeruginosa recombinant ex: protein A, Clostridium perfringens exotoxins, pertussis vaccine (LPF toxoid), tubercular bacilli vaccine, cross-reacting materials (CRM's) which are antigenically similar to bacterial toxins but are non-toxic by means of mutation, preferably CRM 197 obtained according to Pappenheimer, et al., *Immunochemistry*, 9, 891-906 (1972) and other bacterial protein carriers, for example meningococcal outer membrane protein. When a vaccine is being prepared the substrate protein can itself be an immunogen. Further substrate materials include immunogenic proteins derived by bacteria such as .beta.-hemolytic streptococci, Haemophilus influenza, meningococci, pneumococci and E. coli. Other substrates may also be used in which the substrate has been modified to contain a chemically linked amino group, for example polysaccharides to which an aminoalkyl group is attached through a covalent linkage.

Detailed Description Paragraph Type O (12):

[12] Szu, S. C., X. Li, R. Schneerson, J. H. Vickers, D. Bryla, and J. B. Robbins. 1989. Comparative immunogenicities of Vi polysaccharide-protein conjugates composed of cholera toxin or its B subunit as a carrier bound to high- or lower-molecular-weight Vi. *Infect. Immun.* 57:3823-3827.

Detailed Description Paragraph Type O (13):

[13] Szu, S. C., X. Li, A. L. Stone, and J. B. Robbins. 1991. Relation between structure and immunologic properties of the Vi capsular polysaccharide. *Infect. Immun.* 59:4555-4561.

Detailed Description Paragraph Type O (14):

[14] Szu, S. C., A. L. Stone, J. D. Robbins, R. Schneerson, and J. B. Robbins. 1987. Vi capsular polysaccharide-protein conjugates for prevention of typhoid fever. *J. Exp. Med.* 166:1510-1524.

Detailed Description Paragraph Type O (15):

[15] Szu, S. C., D. N. Taylor, A. C. Trofa, J. D. Clements, J. Shiloach, J. C. Sadoff, D. A. Bryla and J. B. Robbins. 1994. Laboratory and preliminary clinical characterization of Vi capsular polysaccharide-protein conjugate vaccines. *Infect. Immun.* (in press).

CLAIMS:

13. A composition having the general formula (VI) in which G is the radical of a glycoside wherein the glycoside is a mono, oligo or polysaccharide and n and m are each independently an integer of from 1 to 12, R.sub.1 and R.sub.2 are each independently H, lower alkyl, a hydroxyl group, R.sub.4 and R.sub.5 are each independently H, lower alkyl or a hydroxyl group, and R.sub.6 is the residue of an amine group-containing carrier.

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L7: Entry 12 of 36

File: USPT

Apr 14, 1998

DOCUMENT-IDENTIFIER: US 5738855 A

TITLE: Synthesis of typhoid fever vaccine from a plant or fruit polysaccharide

Abstract Paragraph Left (1):

The present invention is a modified plant, fruit or synthetic oligo- or polysaccharide which has been structurally altered so as to render the modified saccharide antigenically similar to the Vi of Salmonella typhi. The modified saccharide may be conjugated to a carrier to form a conjugate that is immunogenic against S. typhi. Antibodies produced in response to the immunogenic conjugate are protective against typhoid fever. Methods are provided for making the modified saccharide and the immunogenic conjugate.

Brief Summary Paragraph Right (2):

Typhoid fever, caused by Salmonella typhi, remains a common and serious disease in many parts of the world. The capsular polysaccharide (Vi) is both an essential virulence factor and a protective antigen of Salmonella typhi [19]. Tacket et al. in J. Infect. Dis. 154:342-345 (1986) disclose a vaccine made from the Vi capsular polysaccharide of Salmonella typhi. Field trials in Nepal and in the Republic of South Africa showed that a single injection of Vi conferred about 70% protection against typhoid fever in older children and in adults [1,13]. The mechanism of its protective action, similar to other polysaccharide vaccines, is to elicit a critical level of serum antibodies.

Brief Summary Paragraph Right (3):

The immunologic properties of the Vi that limits its use as a vaccine are: 1) only about 70% efficacy in individuals 5 to 45 years of age; 2) an age-dependent serum antibody response, Vi elicited a comparatively short-lived antibody responses in 2 to 5 year old children and only low levels of antibodies in a fraction of children <2 years-old and; 3) reinjection did not elicit a booster antibody response (T-cell independent) [15,19]. To increase its immunogenicity and to induce T-cell dependence, the Vi was conjugated to proteins [22,24,25]. A clinical trial in adults in the United States showed that Vi-protein conjugates elicited significantly higher levels of serum antibodies than the Vi alone [25].

Brief Summary Paragraph Right (4):

The Vi is a linear homopolymer of (1.fwdarw.4)-alpha-D-GalApNAc, variably O-acetylated at C.sub.3 (FIG. 1) [19, 23]. Whiteside and Baker in J. Immunol. 86:538-542 (1961) and Landy et al., Am. J. Hyg. 73:55-65 (1961) disclose that the O-acetyl groups on Vi is essential for its antigenicity. Szu et al. disclose a conjugate scheme for Vi capsular polysaccharide covalent bound to a carrier protein (22, 23, 24). However, synthesis of Vi-protein conjugates poses several problems. First, the high molecular weight of Vi (about 2.times.10.sup.3 kD) causes conjugates to be poorly soluble. Second, standardization of Vi conjugates has been hindered by a lack of a colorimetric method for quantification of this polysaccharide [21]. Colorimetric methods are not applicable to the Vi because the polyhexosaminuronic acid structure resists acid hydrolysis and does not form

a chromophore in the carbazole assay.

Brief Summary Paragraph Right (5):

Szewczyk and Taylor in Infect. Immun. 29:539-544 (1980) taught the art of O-acetylated polygalacturonic acid to form a compound that is antigenically indistinguishable from the Vi as determined by immunodiffusion. The O-acetylated pectin, even though antigenic, is not immunogenic in vivo. Avery and Goebel in J. Exp. Med. 50:531 (1929) and Goebel in J. Exp. Med. 50:469-520 (1929) showed that the immunogenicity of pneumococcus type 3 polysaccharide could be increased by binding it chemically to a carrier protein. This principle has been applied successfully to increase the immunogenicity of capsular polysaccharides of other pathogens (7, 10, 22, 24).

Brief Summary Paragraph Right (8):

It is a further object of the present invention to produce an antigen based on a plant, fruit or synthetic oligo- or polysaccharide which is immunologically similar to the Vi antigen of Salmonella typhi. Preferably the oligo- or polysaccharide is based on pectin which has been modified by acetylation at the C.sub.2 and/or C.sub.3 hydroxyls of its galacturonate subunit.

Brief Summary Paragraph Right (9):

It is yet another object of the present invention to provide an immunogen that elicits antibodies that bind Vi of S. typhi in which the immunogen is based on a plant, fruit or synthetic oligo- or polysaccharide conjugated with a carrier.

Brief Summary Paragraph Right (10):

It is yet another object of the present invention to provide antibodies against Vi of S. typhi which are elicited by immunization with a plant, fruit or synthetic polysaccharide-carrier conjugate.

Brief Summary Paragraph Right (11):

According to the present invention, methods are provided to synthesize a modified plant, fruit or synthetic oligo- or polysaccharide which is structurally similar to the Vi antigen.

Drawing Description Paragraph Right (1):

FIG. 1 shows the structure of the repeating unit of the Vi, the pectin and the O-acetylated pectin. For Vi, C.sub.2 (R) is N-acetylated and C.sub.3 (R.sup.1) is O-acetylated; for pectin, C.sub.2 and C.sub.3 are hydroxylated; for OAcPec, C.sub.2 and C.sub.3 are O-acetylated, n=number of subunits.

Drawing Description Paragraph Right (4):

FIG. 4 shows the antigenicity of the O-acetylated pectin compared with Vi by double immunodiffusion. Center well, B-260 Vi antiserum, 1) Vi, 100 .mu.g/ml; 2) OAcPec K.sup.+ form; 3) OAcPec Ca.sup.++ form; 4) OAcPec C.sub.2 H.sub.5 N.sup.+ form.

Drawing Description Paragraph Right (5):

FIG. 5 shows the quantitative precipitin analysis of pectin (.DELTA.), OAcPec (o) and Vi (O).

Drawing Description Paragraph Right (6):

FIG. 6 shows the temperature dependent stability of O-acetyls on Vi at 4.degree. C. (0--0), Vi at 22.degree. C. (quadrature--quadrature), Vi at 37.degree. C. (.DELTA.--.DELTA.), Vi at 60.degree. C. (.diamond.--.diamond.), OAcPec at 4.degree. C. (.circle-solid.--.circle-solid.), OAcPec at 22.degree. C. (.box-solid.--.box-solid.), OAcPec at 37.degree. C. (.tangle-solidup.--.tangle-solidup.) and OAcPec at 60.degree. C. (.diamond-solid.--.diamond-solid.). The decrease in extent of O-acetylation is depicted as the % remaining after incubation at the various intervals and temperatures compared to the starting material.

Detailed Description Paragraph Right (1):

The Vi molecule of Salmonella typhi has a simple structure which is a linear polysaccharide having repeating sugar subunits. The antigenicity and immunogenicity of Vi depends on its N-acetyl at C.sub.2 and O-acetyl at C.sub.3 on each galacturonate subunit [19,23]. As shown for Vi and other polysaccharides, removal of the O-acetyls removed most of the antigenicity and all of the immunogenicity of the Vi [23,26]. The precise role of N-acetyl is not known as selective removal of the N-acetyl on Vi has not been accomplished. The present invention mimics the simple structure of Vi by modification of plant, fruit or synthetic saccharides. The modified plant, fruit or synthetic saccharides resemble Vi in antigenic and immunogenic properties and as such they have the capacity to act as an effective vaccines against typhoid fever.

Detailed Description Paragraph Right (2):

The present invention encompasses a modified plant, fruit or synthetic oligosaccharide or polysaccharide. Oligosaccharide as defined herein is a carbohydrate containing from two to ten simple sugar subunits linked together. A polysaccharide as defined herein is a carbohydrate containing more than ten simple sugar subunits linked together. The present invention preferably encompasses a modified pectin or modified D-galacturonan, oligogalacturonate or polygalacturonate and mixtures thereof. As used herein, modified pectin or modified oligogalacturonate or polygalacturonate refers to native or naturally occurring pectin or synthetic D-galacturonan, oligogalacturonate and polygalacturonate that has been structurally altered. Such structural alterations are any alterations that render the modified pectin or modified D-galacturonan, oligogalacturonate or polygalacturonate antigenically similar to the Vi antigen of Salmonella typhi. The structural alterations substantially approximate the structure of the Vi antigen of S. typhi.

Detailed Description Paragraph Right (3):

Preferably, a modified pectin, D-galacturonan, oligo-, and polygalacturonate of this invention is further characterized by its ability to immunologically mimic an epitope (antigenic determinant) expressed by S. typhi. Such a modified pectin, D-galacturonan, oligo-, and polygalacturonate is useful herein as a component in an inoculum for producing antibodies that immunoreact with S. typhi, and preferably immunoreact with the Vi of S. typhi.

Detailed Description Paragraph Right (4):

As used herein, the phrase "immunologically mimic" in its various grammatical forms refers to the ability of a modified pectin, modified D-galacturonan, oligogalacturonate and polygalacturonate of this invention to immunoreact with an antibody of the present invention that recognizes and binds to a native epitope on the Vi of S. typhi as defined herein.

Detailed Description Paragraph Right (5):

It should be understood that a subject modified pectin, modified D-galacturonan, oligogalacturonate and polygalacturonate need not be structurally identical to the Vi antigen so long as it includes the required sterical structure and is able to elicit antibodies that react with the Vi antigen on S. typhi.

Detailed Description Paragraph Right (6):

A subject modified pectin, modified D-galacturonan, oligogalacturonate and polygalacturonate includes any substituted analog, fragment or chemical derivative of a pectin so long as the modified pectin, modified D-galacturonan, oligogalacturonate and polygalacturonate is capable of reacting with antibodies that react with the Vi antigen. Therefore, a present modified pectin, modified D-galacturonan, oligogalacturonate and polygalacturonate can be subject to various changes that provide for certain advantages in its use.

Detailed Description Paragraph Right (10):

The Vi molecule has N-acetyl groups at position C.sub.2 and O-acetyl groups at position C.sub.3. If all of the

C.sub.2 positions have acetyl groups and all the C.sub.3 positions on Vi contain acetyl groups, then by definition, the Vi molecule is theoretically 200% fully acetylated. In most preparations of Vi the percent acetylation varies. The C.sub.2 position is usually about 100% N-acetylated and the C.sub.3 position is from about 60-90% O-acetylated depending on normal variation in preparations of Vi. The modified pectin, D-galacturonan, oligogalacturonate and polygalacturonate of the present invention approximates the total percent acetylation of Vi.

Detailed Description Paragraph Right (12):

In one embodiment, the modified pectin and the modified D-galacturonan, oligogalacturonate and polygalacturonate of the present invention has a molar ratio of O-acetyl groups/mole galacturonan sufficient to elicit antibodies that bind to Vi. The molar ratio may be at least 0.5 mole of O-acetyl/mole galacturonan (Gal A), preferably at least 1.6 moles O-acetyl/mole Gal A, more preferably between about 1.6 and about 1.9 moles O-acetyl/mole Gal A. In one embodiment, the ratio is about 1.9 moles O-acetyl/mole Gal A.

Detailed Description Paragraph Right (14):

As with other polysaccharides, the molecular weight of the Vi alone and as a Vi-carrier conjugate is related to its immunogenicity [16, 17, 22]. Thus, the modified pectin and modified D-galacturonan, oligogalacturonate and polygalacturonate may vary in molecular weight in order to enhance its antigenicity or to enhance its immunogenicity when in a conjugate form. The modified pectin and modified D-galacturonan, oligogalacturonate and polygalacturonate may have from about 2 to about 1,000 modified galacturonic subunits, preferably from about 50 to about 800, more preferably from about 200 to about 600 monosaccharide subunits. The molecular weight of the modified pectin may range from about 100 to about 1,000,000, preferably from about 200,000 to about 600,000. In one embodiment the molecular weight of the modified pectin is approximately 400 kD.

Detailed Description Paragraph Right (15):

In addition to the modifications of the galacturonic acid at position C.sub.2 or C.sub.3, other substitutions or deletions are encompassed, such that the substitutions or deletions result in a modified pectin and modified D-galacturonan, oligogalacturonate and polygalacturonate that is antigenically similar to the Vi antigen of *S. typhi*.

Detailed Description Paragraph Right (16):

In one particular embodiment, naturally occurring pectin is modified as to replace the hydroxyl groups at the C.sub.2 and C.sub.3 positions of galacturonic acid with O-acetyl groups. The modified pectin is referred to herein as OAcPec. The characteristics of OAcPec in comparison with Vi of *S. typhi* is as follows:

Detailed Description Paragraph Right (17):

OAcPec and Vi are antigenically indistinguishable by immunodiffusion (FIG. 4). However, OAcPec, unlike Vi, is not immunogenic in mice probably due to its lower molecular weight [16].

Detailed Description Paragraph Right (18):

Another embodiment of the present invention is a modified pectin, and modified D-galacturonan, oligo-, and polygalacturonate-carrier conjugate. The modified pectin and modified D-galacturonan, oligo-, and polygalacturonate-carrier conjugate is immunogenic to Vi in mammals. By immunogenic is meant that the modified pectin-carrier conjugate and modified D-galacturonan, oligo-, and polygalacturonate-carrier conjugate elicit the production of antibodies upon injection into mammals. The antibodies elicited are capable of specifically reacting or binding to *S. typhi*, are capable of specifically reacting or binding to the Vi of *S. typhi* and are capable of providing passive protection against *S. typhi* in humans. The modified pectin and modified D-galacturonan, oligo-, and polygalacturonate-carrier conjugate of the present invention are capable of inducing a statistically significant rise of antibodies that bind to Vi (booster effect) upon reinjection.

Detailed Description Paragraph Right (19):

Modified pectin, and modified D-galacturonan, oligogalacturonate and modified polygalacturonate have several advantages over the Vi in preparing conjugates for vaccines to prevent typhoid fever. Special P3 facilities are required to culture pathogens such as *S. typhi*. This restricts the availability of Vi and presents safety concerns in preparing a Vi vaccine. The present invention of 1) pectin, D-galacturonan, oligo- and polygalacturonate are easy to obtain, safe and purification is simpler than extraction of the Vi from *S. typhi*; 2) modified oligo- and polygalacturonate can be measured during the synthesis of the conjugate and in the final container by a colorimetric reaction and; 3) there is no solubility problem and the yield of modified pectin, D-galacturonan, oligo- and polygalacturonate-carrier conjugates is higher than with Vi; 4) at the 4.degree. C., the standard storage temperature of vaccines, the stability of modified pectin, D-galacturonan, oligo- and polygalacturonate is similar to that of the Vi.

Detailed Description Paragraph Right (20):

The present invention provides method to prepare a synthetic Vi antigen from a plant, fruit or synthetic oligo- or polysaccharide and to conjugate it with a carrier in order to enhance and elicit a booster response against Salmonella typhi capsular polysaccharide.

Detailed Description Paragraph Right (21):

In one embodiment of the method, pectin, D-galacturonan, oligogalacturonate, or polygalacturonate is O-acetylated at C2 and C3 positions with acetic anhydride. Through carbodiimide condensation the O-acetylated pectin, D-galacturonan, oligogalacturonate, or polygalacturonate is thiolated with cystamine, or aminolated with adipic dihydrazide, diaminoesters, ethyldiamine and the like. Both the thiolated and the aminolated O-acetylated pectin, D-galacturonan, oligogalacturonate, or polygalacturonate are stable, may be freeze dried, and stored in cold. The thiolated intermediate may be reduced and covalently linked to a polymeric carrier containing a sulfhydro group, an N-pyridylthio group. The aminolated intermediate may be covalently linked to a polymeric carrier containing a carboxyl group through carbodiimide condensation. The O-acetylated pectin, D-galacturonan, oligogalacturonate, or polygalacturonate covalently linked to a polymeric carrier is immunogenic in mammals and can serve as a typhoid fever vaccine.

Detailed Description Paragraph Right (24):

Carriers are chosen on the basis of facilitating two functions: 1) to increase the immunogenicity of the polysaccharide and 2) antibodies raised against the carrier are medically beneficial. Carriers that fulfill these criteria are described in the art (7, 10, 22-25). Polymeric carriers can be a natural or a synthetic material containing a primary or/and a secondary amino group, an azido group or a carboxyl group. The carrier can be water soluble or insoluble. Examples of water soluble carriers included but are not limited to natural or synthetic peptides or proteins from bacteria or virus, e.g., tetanus toxin/toxoid, diphtheria toxin/toxoid, Pseudomonas aeruginosa exotoxin/toxoid/protein, pertussis toxin/toxoid, Clostridium perfringens exotoxins/toxoid, and hepatitis B surface antigen and core antigen. Example of water insoluble carriers include but are not limited to are aminoalkyl-Sepharose, e.g., aminopropyl or aminohexyl Sepharose, and aminopropyl glass and the like. Other carriers may be used when an amino or carboxyl group is added through covalent linkage with a linker molecule.

Detailed Description Paragraph Right (26):

The O-acetylated plant, fruit or synthetic D-galacturonan, oligosaccharide or polysaccharide is preferably conjugated to a carrier using a linking molecule. A linker or crosslinking agent, as used in the present invention, is a small linear molecule having a molecular weight of approximately <500 and is non-pyrogenic and non-toxic in the final product form (7, 10, 22-25). To conjugate with a linker or crosslinking agent, either or both of the pectin, D-galacturonan, oligogalacturonate, or polygalacturonate and the carrier are covalently bound to a linker first. The linkers or crosslinking agents are a homobifunctional or heterobifunctional molecules, e.g.,

adipic dihydrazide, ethylene diamine, cystamine, N-succinimidyl-3-(2-pyridylthio) propionate (SPDP), N-succinimidyl N-(2-iodoacetyl)-b-alaninate-propionate (SIAP), succinimidyl 4-(N-Maleimido-methyl) cyclohexane-1-carboxylate (SMCC), 3,3'-dithiodipropionic acid and the like. The linkers are bound to the carboxyl groups of the O-acetylated pectin, D-galacturonan, oligogalacturonate, or polygalacturonate or the carrier through carbodiimide condensation. The linkers are bound to the amino groups of the carrier through carbodiimide condensation or N-hydroxysuccinimidyl ester. The unbound materials are removed by gel filtration or ion exchange column depending on the materials to be separated. The final conjugate consist of the oligo- or polysaccharide and the carrier bound through a linker.

Detailed Description Paragraph Right (27):

Clinical evidence has shown that serum antibodies to the Vi antigen confers immunity to typhoid fever. (1,2). The immunogen used to elicit the antibodies was the Vi capsular polysaccharide. Because Vi antibodies have been shown to be protective against typhoid fever and due to the complexity and safety issues that arise from culturing *S. typhi*, the World Health Organization (WHO) and the U.S. Food and Drug Administration (FDA) no longer require challenge data as criteria for licensing an acellular vaccine against Salmonella typhi (32). WHO and FDA criteria for licensing an acellular vaccine against Salmonella typhi is the demonstration that the preparation elicits Vi antibodies or that the preparation binds to Vi antibodies.

Detailed Description Paragraph Right (28):

The modified pectin-carrier conjugates and modified D-galacturonan, oligogalacturonate and polygalacturonate-carrier conjugates of the present invention elicit antibodies that react with or bind to the Vi antigen. The anti-Vi antibody levels elicited by the modified pectin-carrier conjugates were comparable to those elicited by a Vi-Pseudomonas aeruginosa recombinant exoprotein A (rEPA) conjugate as measured by ELISA. Thus, the modified pectin-carrier and modified D-galacturonan, oligogalacturonate and polygalacturonate-carrier conjugate may be used as an effective vaccine against *S. typhi* to prevent or ameliorate typhoid fever in humans.

Detailed Description Paragraph Right (29):

The present inoculum contains an effective, immunogenic amount of modified pectin-carrier conjugate and modified D-galacturonan, oligogalacturonate and polygalacturonate-carrier conjugates of this invention. The effective amount of modified pectin-carrier conjugate and modified D-galacturonan, oligogalacturonate and polygalacturonate-carrier per unit dose sufficient to induce an immune response to the Vi antigen depends, among other things, on the species of mammal inoculated, the body weight of the mammal and the chosen inoculation regimen as is well known in the art. Inocula typically contain modified pectin-carrier conjugate and modified D-galacturonan, oligogalacturonate and polygalacturonate-carrier conjugate concentrations of oligo- or polysaccharide of about 1 micrograms to about 100 milligrams per inoculation (dose), preferably about 25 micrograms to about 50 milligrams per dose.

Detailed Description Paragraph Right (33):

Testing of the modified pectin-carrier conjugate and modified D-galacturonan, oligogalacturonate, and a polygalacturonate-carrier vaccines is conducted as prescribed by the World Health Organization as described in Example 6 or by any equivalent immunological assay. Elicitation of Vi antibodies is predictive of in vivo efficacy of the conjugates in humans. Antibodies elicited by the modified pectin-carrier conjugates and modified D-galacturonan, oligogalacturonate and polygalacturonate-carrier conjugates are useful in providing passive protection to an individual exposed to *S. typhi* to prevent or ameliorate infection and disease caused by the microorganism.

Detailed Description Paragraph Right (36):

An antibody of the present invention, i.e., an anti-Vi antibody, in one embodiment is characterized as comprising antibody molecules that immunoreact with: 1) *S. typhi* and 2) isolated Vi antigen of *S. typhi*. In

another embodiment the antibody is characterized as comprising antibody molecules that immunoreact with: 1) *S. typhi*, 2) isolated Vi antigen of *S. typhi* and 3) a modified pectin of the present invention, and being substantially free of antibody molecules that immunoreact with native or naturally occurring pectin.

Detailed Description Paragraph Right (38):

The antibodies of the present invention have a number of diagnostic and therapeutic uses. The antibodies can be used as an in vitro diagnostic agent to test for the presence of *S. typhi* in biological samples in standard immunoassay protocols. Such assays include, but are not limited to, radioimmunoassays, EIA, fluorescence assay, Western blot and the like. In one such assay, the biological sample is contacted to antibodies of the present invention and a labelled second antibody is used to detect the presence of *S. typhi*, or the Vi antigen of *S. typhi* to which the antibodies are bound.

Detailed Description Paragraph Right (40):

The antibodies and antigen binding fragments of the present invention are useful in prevention and treatment of infections and diseases caused by *S. typhi* and other microorganisms that have structures immunologically similar to the Vi antigen.

Detailed Description Paragraph Right (46):

Pectin (GENU pectin, from Copenhagen, Denmark, type LM-1912CSZ) was extracted from citrus. Pyrogen-free water (PFW) and pyrogen-free saline (PFS) for clinical use were from Baxter, Deerfield, Wis.; N-succinimidyl 3(2-pyridylidithio) propionate (SPDP) from Pierce, Rockford, Ill.; formamide, cystamine from Fluka, Ronkonkoma, N.Y.; pyridine, NaOH, HCl from Baker Chemical, Philipsburg, N.J., acetic anhydride, dithiothreitol (DTT), EDTA, 1-ethyl-3(d-dimethylaminopropyl) carbodimide (EDAC), acetyl choline, BSA, dithionitrobenzoic acid (Ellman reagent), D-galacturonic acid monohydrate (GalA) and tetrabutylammonium hydroxide from Sigma, St. Louis, Mo.; carbazole from Aldrich, Milwaukee, Wis.; HEPES from Calbiochem, La Jolla, Calif.; bicinchoninic acid (BCA) protein reagent, Sephacyl S-1000, Sephadex G-50, Superose 6 from Pharmacia, Piscataway, N.J. Antiserum to tetanus toxoid (TT) was donated by William Habig, CBER, FDA.

Pseudomonas aeruginosa exotoxin A (ETA) and goat antiserum to this protein were from List Biological Lab., Campbell, Calif., Vi antiserum (B-260) was prepared by multiple intravenous injections of a burro with heat-killed *S. typhi* strain Ty-2[19]. Pseudomonas aeruginosa recombinant exoprotein A (rEPA) was made as described in U.S. Ser. No. 08/189,113 filed Jan. 27, 1994, which is a continuation of U.S. Ser. No. 07/825,089 filed Jan. 24, 1992, abandoned. The Vi-rEPA was made as described in U.S. Pat. No. 5,204,098 issued Apr. 20, 1993.

Detailed Description Paragraph Right (50):

The antigenicity of the O-acetylated pectin was studied by reaction with the antiserum against Salmonella *typhi* in 2-dimensional immunodiffusion using Vi polysaccharide as a comparison. Immunodiffusion was performed in 1% agarose in PBS with B-260 antiserum. Quantitative precipitation was performed with 100 .mu.L of B-260 with equal volumes of antigen, containing 1 to 100 .mu.g/mL, at 37.degree. C. for 1 hour and at 3.degree.-8.degree. C. for five days with occasional mixing. The precipitates were washed in cold PFS three times, dissolved in 0.8% SDS and their A_{sub}.280 recorded [23]. Serum Vi antibodies were measured by ELISA using a pooled hyperimmune mouse sera, quantitated by radioimmunoassay, as the standard [1].

Detailed Description Paragraph Right (51):

The stability of the O-acetyl groups are studied at various temperatures for various periods of time. OAcPec and Vi (1 mg/mL) in PBS, pH 7.0, were incubated at 3.degree.-8.degree. C., 25.degree. C., 37.degree. C. and 60.degree. C. Aliquots were removed at 1, 2, and 12 wks and analyzed for their content of O-acetyl and molecular size by gel filtration.

Detailed Description Paragraph Right (52):

The synthesis of conjugates followed that described for Vi [24]. The polysaccharide (5 mg/mL) was dissolved in 0.2M NaCl. Cystamine (0.1M) was added and the pH adjusted to 5.0 with 0.1M HCl. The temperature was 37.degree. C. for Vi and room temperature for OAcPec. EDAC (0.1M) was added and the reaction mixture stirred for 4 hours with the pH maintained between 4.9 to 5.1. The reaction mixture was dialyzed against PFS with 10 mM phosphate, pH 7, 3.degree.-8.degree. C. for one day, against PFW for 3 days with multiple changes and freeze-dried. Thiolation was measured on an aliquot of the polysaccharides treated with 0.1M DTT at room temperature for 1 hour and passage through a 2.5.times.35 cm P10 column. Void volume fractions were titrated for their sulfhydryl content and the degree of derivatization expressed as percent cystamine.

Detailed Description Paragraph Right (54):

The cystamine-derivatized polysaccharide, 10 mg/mL PBS, pH 7.4, was treated with 0.05M DTT at room temperature for two hours and passed through a 2.5.times.35 cm column of Sephadex G-50 in PBS, pH 7.0. An aliquot was taken to determine its sulfhydryl content and the remainder mixed with an equal weight of SPDP-derivatized protein and stirred at room temperature for 4 hours and at 3.degree.-8.degree. C. overnight. The reaction mixture was passed through a 2.5.times.95 cm column of Sephacryl S-1000 in PFS at 3.degree.-8.degree. C. For the OAcPec-TT, fractions containing protein and polysaccharide were pooled into two batches: OAcPec-TT.sub.1 for the void volume peak and OAcPec-TT.sub.2 for the lower molecular weight fractions. Vi-rEPA was passed through a 2.5.times.95 cm column of Sephacryl S-1000 in PFS and the void volume fractions pooled.

Detailed Description Paragraph Right (56):

The M.sub.r of OAcPec, similar to that of the pectin, had a broad distribution with the major peak about 400 kD (FIG. 3). Unlike pectin, OAcPec was soluble in 0.15M NaCl and did not form a gel in the presence of Ca.sup.++. Molar absorbances in the carbazole assay were 1.32.times.10.sup.3 for OAcPec, 1.61.times.10.sup.3 for pectin and 1.63.times.10.sup.3 for GalA. The differences between pectin and GalA were <2% and are probably due to neutral sugars in the pectin. Vi, in contrast, did not react in the carbazole assay.

Detailed Description Paragraph Right (57):

Pectin did not react with B-260 serum in double immunodiffusion. OAcPec, in contrast, formed a line of identity with Vi (FIG. 4). Precipitation of OAcPec with Vi antiserum did not change with different counter ions including Na.sup.+, Ca.sup.++, K.sup.+ or tetrabutylammonium. At lower degrees of O-acetylation (0.4-0.9 moles O-acetyl/mole GalA) pectin also yielded a line of identity with the Vi (not shown). No precipitation in double immunodiffusion was observed when the O-acetylation of pectin was 1.0 to 0.2 mole/mole GalA. Quantitative precipitation showed that both Vi and OAcPec precipitated 2.6 mg/mL Ab from B-260 antiserum (FIG. 5).

Detailed Description Paragraph Right (58):

The thermostability of the O-acetyls was similar for OAcPec compared to Vi (FIG. 6). Following storage at 3.degree.-8.degree. C. for 12 weeks, there was no change in the concentration of O-acetyls for Vi and OAcPec compared to the original level of O-acetyls for Vi and OAcPec prior to storage: at 22.degree. C., O-acetyls declined to 93% for Vi and to 88% for OAcPec and at 60.degree. C., only 12% of the O-acetyls remained on Vi and 10% on OAcPec.

Detailed Description Paragraph Right (59):

The stabilities of glycosidic linkages of the polysaccharides were studied by gel filtration. There was no change in M.sub.r of OAcPec at 3.degree.-8.degree. C. for three months. After storage of OAcPec at 60.degree. C. for three month, the M.sub.r decreased from 400 kD to 30 kD (not shown). In contrast, the Vi was more stable: little depolymerization was observed after incubation at 60.degree. C. for two weeks and the M.sub.r shifted from 2.times.10.sup.3 kD to 500 kD after 3 months.

Detailed Description Paragraph Right (61):

16-20 g. female. general purpose mice from the NIH colony were injected subcutaneously 1, 2, or 3 times at 2 week intervals with 2.5 .mu.g of the polysaccharide alone or as a conjugate. 10 mice from each group were exsanguinated two weeks after the first injection and one week after the second and third injections. Controls included mice injected with saline, Vi or OAcPec. Vi antibody levels were measured by ELISA with a reference calibrated by RIA.

Detailed Description Paragraph Right (62):

Vi antigen purified from *S. typhi*, alkaline phosphatase labeled goat anti-mouse or alkaline phosphatase labeled anti-human (Kirkegaard & Perry Lab. Inc.)=Conjugate; -p- nitrophenyl phosphate disodium (Sigma Fine Chemical)=Substrate; bovine serum albumin (BSA) (Sigma Fine Chemical); sodium carbonate (Na₂CO₃); sodium bicarbonate (NaHCO₃); sodium chloride; Brij 35; Na N₃; Tris-HC-MgCl₂; HCl; PBS.

Detailed Description Paragraph Right (68):

1. Store frozen Vi polysaccharide (0.1 mg/ml) 500 .mu.l aliquots.

Detailed Description Paragraph Right (69):

2. Coat microtiter plates (96 well, flat bottom, polystyrene Immunolon microtiter plates) with Vi. Dilute 1 to 2 .mu.g Vi per ml in "coating buffer" use 100 .mu.l/well, shake gently. Incubate plates at 4.degree. C. overnight covered with polyester film.

Detailed Description Paragraph Right (80):

As reported, Vi elicited serum antibodies in mice after one injection and reinjection did not elicit a booster response [14,22-25]. Neither the pectin nor the OAcPec elicited Vi antibodies after any injections. After one injection, the Vi and OAcPec conjugates elicited similar levels of antibodies. Following the second injection, the conjugates elicited a booster response (P<0.001) with the geometric mean antibody levels highest for Vi-rEPA (17.1) >OAcPec-TT₂ (7.65) >OAcPec-TT₁ (5.47). These differences, however, were not statistically significant. The third injection of all 3 conjugates did not elicit a booster response. Lastly, there were no statistically significant differences in the geometric mean (GM) Vi antibody levels elicited by OAcPec-TT₁ and OAcPec-TT₂ after any of the three injections.

Detailed Description Paragraph Right (81):

The modified pectin, modified D-galacturonan, oligogalacturonate and polygalacturonate-carrier conjugates vaccines are tested as per WHO requirements for acellular vaccines against Salmonella typhi (32).

Detailed Description Paragraph Right (83):

Those lots fulfilling the WHO requirements are suitable for use in humans as a Salmonella typhi vaccine.

Detailed Description Paragraph Right (84):

Volunteers between 18 and 45 years of age, who have no antibodies to hepatitis B and to HIV-1 are recruited. Following receipt of their informed consent, volunteers receive 1 injection of Vi (25 .mu.g in 0.5 mL) (1,13) or 1 injection of a modified pectin-carrier conjugate (25 .mu.g polysaccharide in 0.5 mL) of the present invention intramuscularly. Oral temperature is taken and the injection site of each volunteer is inspected 6, 24 and 48 hours after each injection. Volunteers receive a second injection at 6 weeks and are bled 2 weeks later and 26 weeks after the first injection. Antibodies reactive to Vi are determined by ELISA as described herein.

Detailed Description Paragraph Left (7):

Stability of OAcPec and Vi O-acetyls

Detailed Description Paragraph Left (14):

Vi antibodies:Detailed Description Paragraph Center (12):

ELISA Procedure for the Measurement of Vi Antibody

Detailed Description Paragraph Type 0 (4):

4. Bystricky, S., and S. C. Szu. 1994. O-acetylation affects the binding properties of the carboxyl groups on the Vi bacterial polysaccharide Biophys. Chem. 51:1-7.

Detailed Description Paragraph Type 0 (9):

9. Fass, R., M. van de Walle, A. Shiloach, A. Joslyn, J. Kaufman, and J. Shiloach. 1991. Use of high density cultures of *Escherichia coli* for high level production of recombinant Pseudomonas aeruginosa exotoxin A. Appl. Microbiol. Biotechnol. 36:65-69.

Detailed Description Paragraph Type 0 (11):

11. Heidelberger, M., and P. A. Rebers. 1960. Immunochemistry of the pneumococcal types II, V, and VI. I. The relation of type VI to type II and other correlations between chemical constitution and precipitation in antisera to type VI. J. Bacteriol. 80:145-153.

Detailed Description Paragraph Type 0 (13):

13. Klugman, K. P., H. J. Koornhof, I. T. Gilbertson, J. B. Robbins, R. Schneerson, D. Schulz, M. Cadoz, J. Armand, Vaccine Advisory Committee. 1987. Protective activity of Vi capsular polysaccharide vaccine against typhoid fever. Lancet ii:1165-1169.

Detailed Description Paragraph Type 0 (14):

14. Landy, M. 1957. Studies on the Vi antigen. VII. Characteristics of the immune response in the mouse. Am. J. Hyg. 65:81-93.

Detailed Description Paragraph Type 0 (15):

15. Landy, M. 1954. Studies in Vi antigen. VI. immunization of human beings with purified Vi antigen. Amer. J. Hyg. 60:52-62

Detailed Description Paragraph Type 0 (16):

16. Martin, D. G., F. G. Jarvis and K. C. Milner. 1967. Physicochemical and biological properties of sonically treated Vi antigen. J. Bacteriol. 94:1411-1416.

Detailed Description Paragraph Type 0 (19):

19. Robbins, J. D., and J. B. Robbins. 1984. Protective role of the Vi capsular polysaccharide (Vi antigen) of Salmonella typhi. J. Infect. Dis. 47:436-499

Detailed Description Paragraph Type 0 (21):

21. Stone, A. L., and S. C. Szu. 1988. Application of optical properties of Vi capsular polysaccharide for quantitation of the Vi antigen in vaccines for typhoid fever. J. Clin. Microbiol. 26:719-725.

Detailed Description Paragraph Type 0 (22):

22. Szu, S. C., X. Li, R. Schneerson, J. H. Vickers, D. Bryla, and J. B. Robbins. 1989. Comparative immunogenicities of Vi polysaccharide-protein conjugates composed of cholera toxin or its B subunit as a carrier bound to high- or lower-molecular-weight Vi. Infect. Immun. 57:3823-3827.

Detailed Description Paragraph Type 0 (23):

23. Szu, S. C., X. Li, A. L. Stone, and J. B. Robbins. 1991. Relation between structure and immunologic properties of the Vi capsular polysaccharide. *Infect. Immun.* 59:4555-4561.

Detailed Description Paragraph Type 0 (24):

24. Szu, S. C., A. L. Stone, J. D. Robbins, R. Schneerson, and J. B. Robbins. 1987. Vi capsular polysaccharide-protein conjugates for prevention of typhoid fever. *J. Exp. Med.* 166:1510-1524.

Detailed Description Paragraph Type 0 (25):

25. Szu, S. C., D. N. Taylor, A. C. Trofa, J. D. Clements, J. Shiloach, J.C. Sadoff, D.A. Bryla and J. B. Robbins. 1994. Laboratory and preliminary clinical characterization of Vi capsular polysaccharide-protein conjugate vaccines. *Infect. Immun.* (in press).

Detailed Description Paragraph Type 0 (26):

26. Szewczyk, B., and A. Taylor. 1980. Immunochemical properties of Vi antigen from Salmonella typhi Ty2: Presence of two antigenic determinants. *Infect. Immun.* 29:539-544.

Detailed Description Paragraph Type 0 (29):

29. Gaines, S., J. A. Currie and J. G. Tully, 1960. Production of incomplete Vi antibody in mice. *Proc. Soc. Exp. Biol. Med.* 104:602.

Detailed Description Paragraph Type 0 (30):

30. Gaines, S., J. A. Currie and J. G. Tully, 1965. Production of incomplete Vi antibody in man by typhoid vaccine. *Am. J. Epidemiol.* 81:350.

Detailed Description Paragraph Type 0 (31):

31. Kawata, Y. 1970. A study of the molecular types of immunoglobulin. II. Mouse protection study Vi antibody against typhoid infection. *Acta Medicine Univ. Kioto* 40: 284.

Detailed Description Paragraph Type 0 (32):

32. World Health Organization. 1994. Annex 1 Requirements for Vi polysaccharide typhoid vaccine (Requirements for Biological Substances No. 48) WHO Technical Report Series No. 840:14-33.

Detailed Description Paragraph Type 1 (1):

1) the M.sub.1 of Vi (.about.2.times.10.sup.3 kD) is higher than that of OAcPec (.about.400 kD); 2) the N-acetyl at C.sub.2 in the Vi is replaced by an O-acetyl in OAcPec and; 3) OAcPec has <5% neutral sugars and Vi had a nondetectable amount. At 3.degree.-8.degree. C., the stability of OAcPec as measured by its O-acetyl content and molecular size, is similar to that of Vi. At higher temperatures, the molecular size of Vi is more stable than the OAcPec probably due to the stabilizing effect of a hydrogen bond between the N-acetyl and the carboxyl of the adjacent residue [23]. Since vaccines will be stored at 1toreq.3.degree.-8.degree. C., the stability characteristic of OAcPec and Vi can be considered as similar.

Detailed Description Paragraph Type 1 (2):

1) serological testing for immunological identify with a standardized Vi antigen; 2) polysaccharide content; 3) sterility testing; 4) pyrogenicity testing; 5) toxicity testing; 6) preservative content (if added); 7) pH; and 8) stability studies.

Detailed Description Paragraph Table (1):

TABLE 1 _____ Composition of conjugates of O-acetylated pectin (OAcPec) with tetanus toxoid (TT) and Vi with Pseudomonas aeruginosa recombinant Exoprotein A (rEPA). M.sub.r Ps Cysteamine/ SPDP/protein Ps/protein Conjugate (kD) PS (%) molar ratio ratio (wt/wt)

OAcPect-TT.sub.1 400 4.0 3.6 0.4 OAcPect-TT.sub.2 400
 4.0 3.6 0.8 Vi-rEPA 2 .times. 10.sup.3 1.3 2.1 0.2

Detailed Description Paragraph Table (2):

TABLE 2 Vi antibodies (.mu.g Ab/mL serum) in mice immunized with Vi, Vi-rEPA, Pectin, O-acetyl Pectin (OAcPec) and OAcPec-TT conjugates. Geometric Mean [n = 10] Immunogen 1st Injection 2nd Injection 3rd Injection
Vi 0.65 0.76 Not Done Vi-rEPA 0.85.sup.a 17.1.sup.b 12.7.sup.c Pectin <0.03 <0.03 Not Done OAcPec <0.03 0.04
Not Done OAcPec-TT.sub.1 0.98.sup.d 5.47.sup.e 6.29.sup.f OAcPec-TT.sub.2 0.87.sup.g 7.65.sup.h 5.29.sup.i
b, c vs a, P = 0.0002, f, e vs d, P = 0.0001, h vs g, P 0.0002, i vs g, P = 0.007, b vs c, f vs e, h vs i, b vs e or h, c vs f, i, NS.

Other Reference Publication (7):

Acharya et al, Prevention of Typhoid Fever in Nepal with the Vi Capsular Polysaccharide of *Salmonella typhi*, New England Journal of Medicine, 317:1101-1105, Oct. 29, 1987.

Other Reference Publication (10):

Landy et al, Studies On Vi Antigen VIII. Role of Acetyl in Antigenic Activity, Am. J. Hvg., 1961, vol. 73, pp. 55-65.

Other Reference Publication (11):

Whiteside et al, The Vi Antigens of the Enterobacteriaceae V. Serologic Differences of Vi1 Antigens Revealed by Deacetylation, J. Immunol., Sep. 21, 1961, vol. 86, pp. 538-542.

Other Reference Publication (12):

Szewczyk et al, Immunochemical Properties of Vi Antigen From *Salmonella typhi* TY2: Presence of Two Antigenic Determinants, Infection and Immunity, Aug. 1980, pp. 539-544, vol. 29, No. 2.

Other Reference Publication (13):

Szu et al, Vi Capsular Polysaccharide-Protein Conjugates for Prevention of Typhoid Fever, Journal of Experimental Medicine, vol. 166, Nov. 1987, pp. 1510-1524.

Other Reference Publication (14):

Szu et al, Laboratory and Preliminary Clinical Characterization of Vi Capsular Polysaccharide-Protein Conjugate Vaccines, Infection and Immunity, vol. 62 (Oct.), pp. 4440-4444, 1994.

Other Reference Publication (15):

Robbins, J.D. et al, Re-Examination of Protective Role of the Vi Capsular Polysaccharide (Vi Antigen) of *Salmonella typhi*, J. Infect. Disease, vol. 150 (No. 3), pp. 436-499, 1984.

CLAIMS:

1. A method to prepare an immunogenic modified plant, fruit or synthetic oligogalacturonate or polygalacturonate-carrier conjugate against Salmonella typhi comprising:

(a) O-acetylating a plant, fruit or synthetic oligogalacturonate or a polygalacturonate to form a modified plant, fruit or synthetic O-acetylated oligogalacturonate or a modified plant, fruit or synthetic O-acetylated polygalacturonate,

(b) conjugating the modified plant, fruit or synthetic O-acetylated oligogalacturonate or the modified plant,

fruit or synthetic O-acetylated polygalacturonate to a carrier to form the modified plant, fruit or synthetic oligogalacturonate or modified plant, fruit or synthetic polygalacturonate-carrier conjugate which is immunogenic against Salmonella typhi.

10. The method of claim 1 wherein the carrier is a protein selected from the group consisting of bacterial protein, viral protein, tetanus toxoid, tetanus toxin, diphtheria toxin, Pseudomonas aeruginosa exotoxin, Pseudomonas aeruginosa toxoid, pertussis toxin, pertussis toxoid, Clostridium perfringens exotoxin, Clostridium perfringens toxoid, hepatitis B surface antigen, hepatitis B core antigen and Pseudomonas exoprotein A.

14. The method of claim 13 wherein the agent is selected from the group consisting of N-succinimidyl 3-(2-pyridyldithio) propionate, adipic dihydrazide, cystamine, 3,3' dithiodipropionic acid, ethylene diamine, N-(2-iodoacetyl)-b-alaninate-propionate and succinimidyl 4-(N-Maleimido-methyl) cyclohexane-1-carboxylate.

25. A chemically modified saccharide-carrier conjugate comprising a plant, fruit or synthetic saccharide chemically modified by O-acetylation covalently linked to a carrier, said conjugate elicits antibodies which are immunoreactive with Salmonella typhi, Vi capsular polysaccharide of Salmonella typhi, and the modified saccharide.

35. The modified plant, fruit or synthetic saccharide-carrier conjugate of claim 25 wherein the carrier is selected from the group consisting of bacterial protein, viral protein, tetanus toxoid, diphtheria toxin, Pseudomonas aeruginosa exotoxin, Pseudomonas aeruginosa toxoid, pertussis toxin, pertussis toxoid, Clostridium perfringens exotoxin, Clostridium perfringens toxoid, hepatitis B surface antigen, hepatitis B core antigen and Pseudomonas exoprotein A.

38. The modified plant, fruit or synthetic saccharide-carrier conjugate of claim 37 wherein the crosslinking agent is selected from the group consisting of adipic dihydrazide, ethylene diamine, cystamine, N-succinimidyl-3-(2-pyridyldithio) propionate, N-succinimidyl N-(2-iodoacetyl)-b-alaninate-propionate, succinimidyl 4-(N-Maleimido-methyl) cyclohexane-1-carboxylate, and 3,3'-dithiodipropionic acid.

39. An immunogen against Salmonella typhi comprising: a plant, fruit or synthetic saccharide modified by O-acetylation covalently linked to a carrier, said modified saccharide-carrier conjugate elicits antibodies in mammals, said antibodies are specifically immunoreactive against Vi of Salmonella typhi.

40. The immunogen of claim 39 wherein the modified plant, fruit or synthetic saccharide has immunological identity with the Vi of Salmonella typhi as measured by immunodiffusion.

44. The immunogen of claim 39 wherein the carrier is selected from the group consisting of bacterial protein, viral protein, tetanus toxoid, diphtheria toxin, Pseudomonas aeruginosa exotoxin, Ps. aeruginosa toxoid, pertussis toxin, pertussis toxoid, Clostridium perfringens exotoxin, Clostridium perfringens toxoid, hepatitis B surface antigen, hepatitis B core antigen and Pseudomonas exoprotein A.

49. A method of actively immunizing a human against typhoid fever comprising: administering in vivo a sufficient amount of an O-acetylated plant, fruit or synthetic saccharide linked to a carrier, said amount is sufficient to elicit antibody that binds to Vi of Salmonella typhi.